

Protein-protein interaction investigated by steered molecular dynamics simulation

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Swiss Institute of Bioinformatics

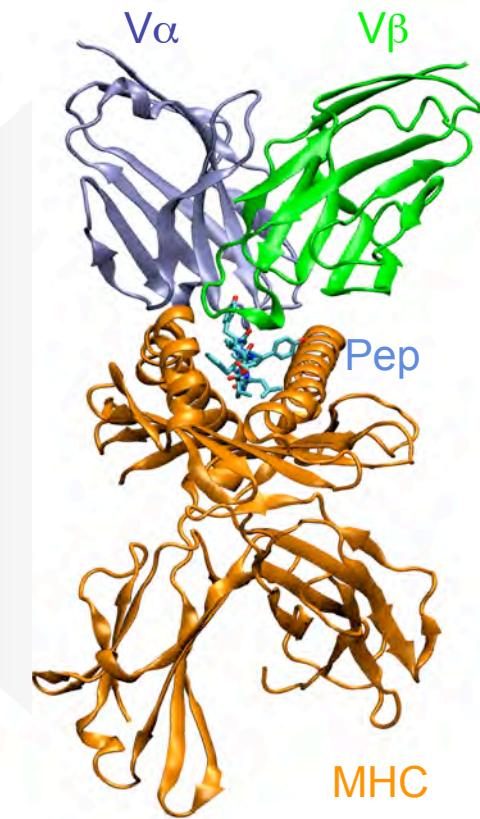
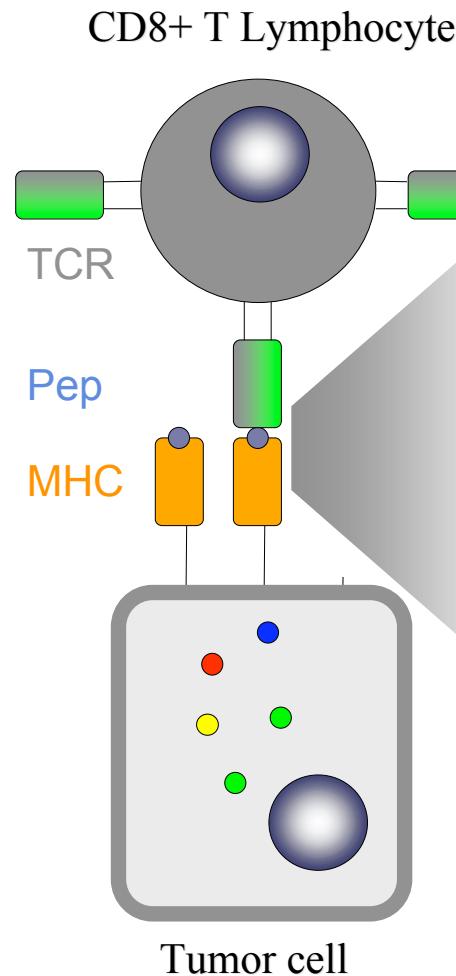
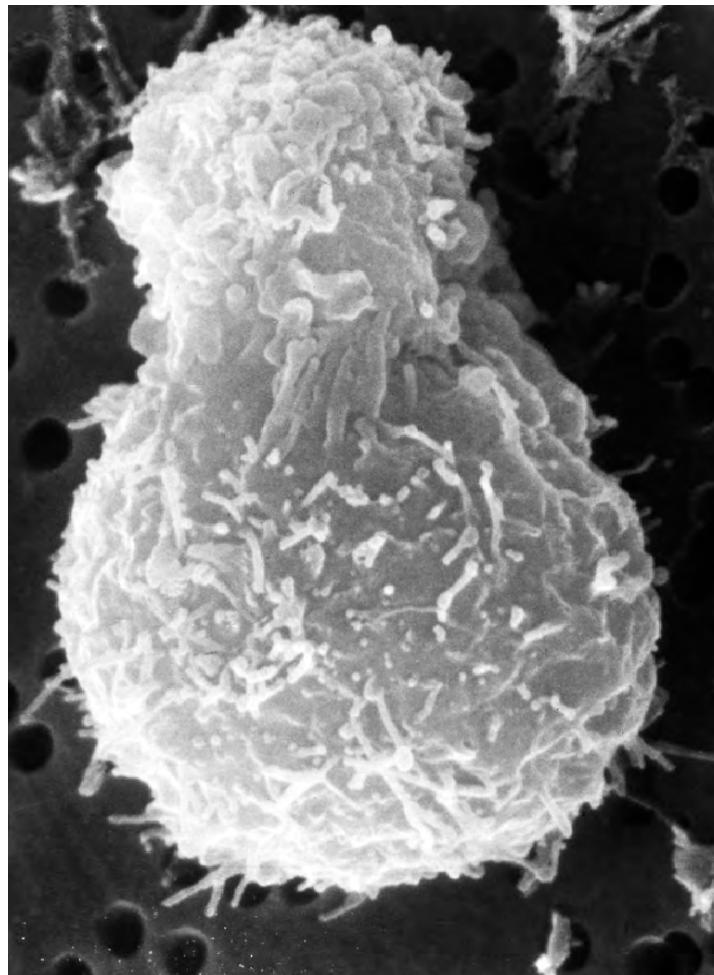
Lausanne

Switzerland



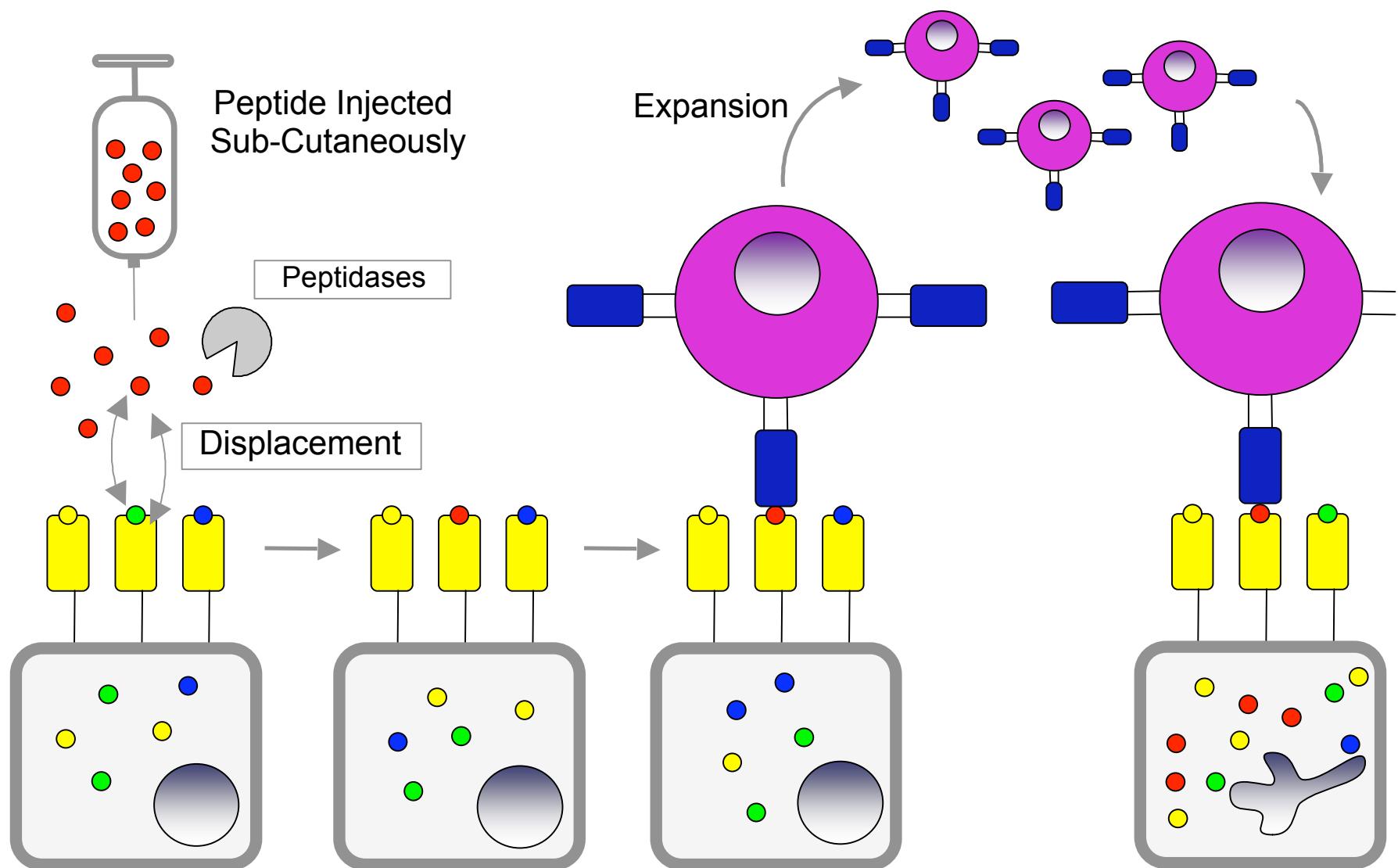
Sandia National Lab,
Albuquerque, NM
20.11.2008

Recognition by CD8+ T lymphocyte



X-ray structure of
bound TCR-p-MHC
(Garboczi & al. *Nature* 96)

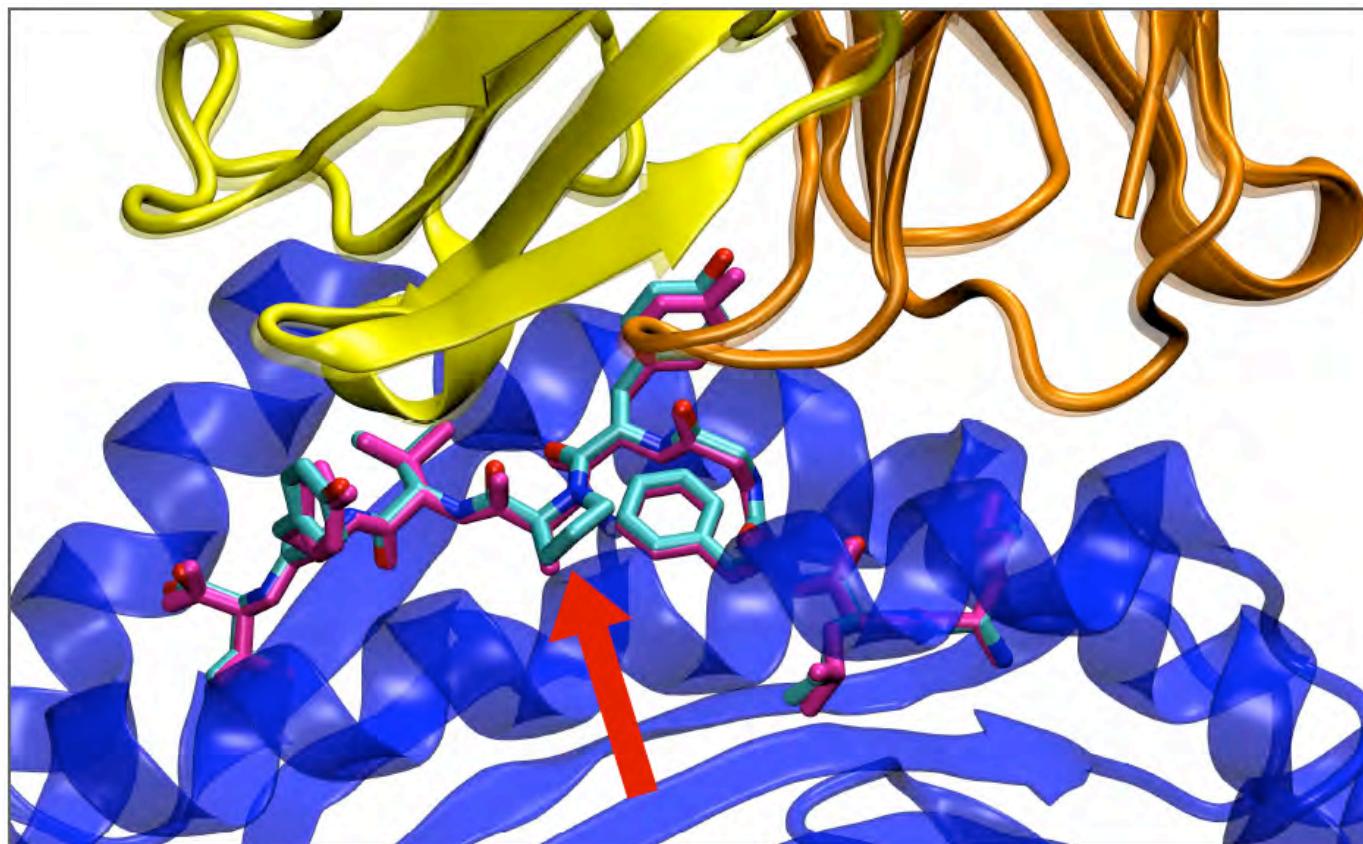
Vaccination principle in cancer immunotherapy





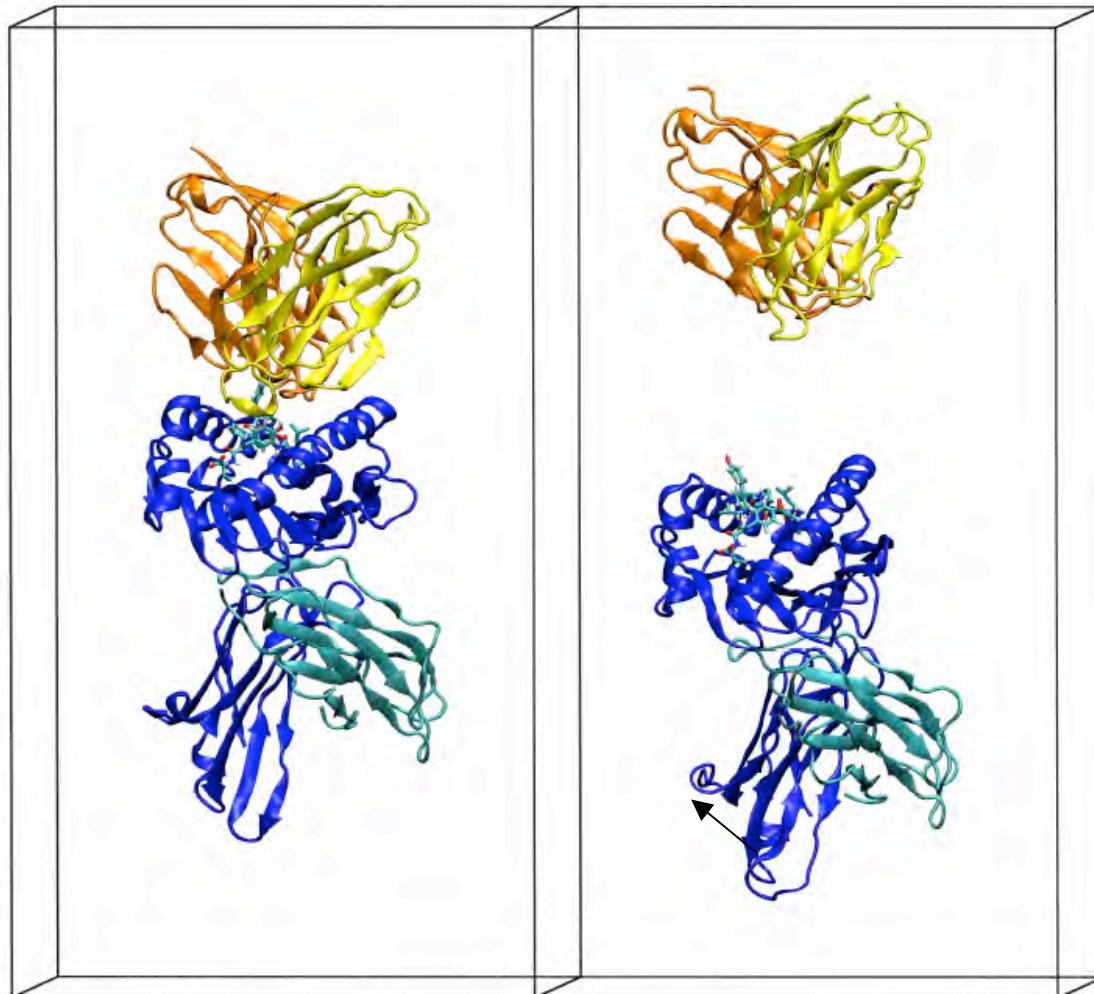
Peptide mutant : Pro6 → Ala

- Affinity reduced from 1.1 μM to 116 μM
- Cytotoxic activity lost
- Very similar crystal structures

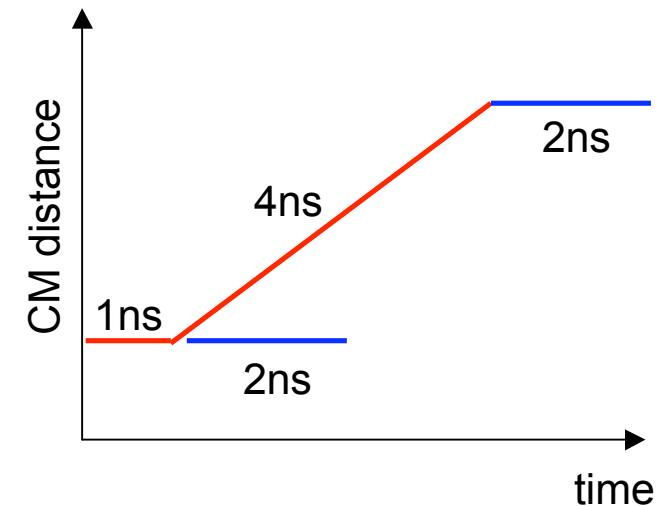


Y. H. Ding et al., Immunity 11 (1999), no. 1, 45.

Steered + equilibrium MD

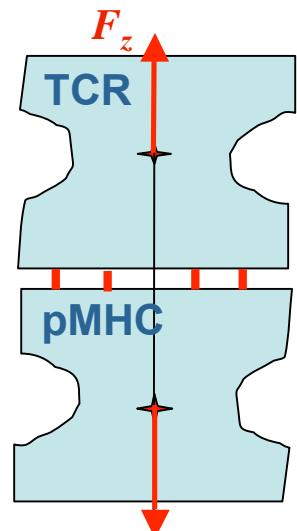


- 605 aa, 85'000 atoms
- gromos 43a1 force field
- gromacs 3.3



- 150 times, with different initial velocities
- 1.35 μ s total

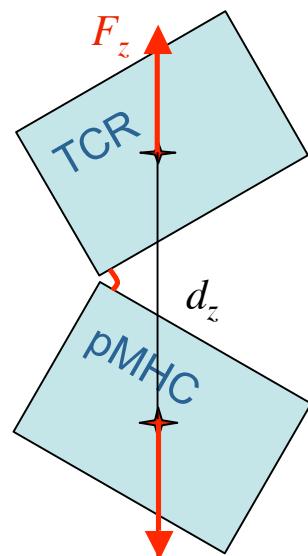
Uniform pulling vs. Individual Pulling



Stretching / unfolding

Considered improbable :

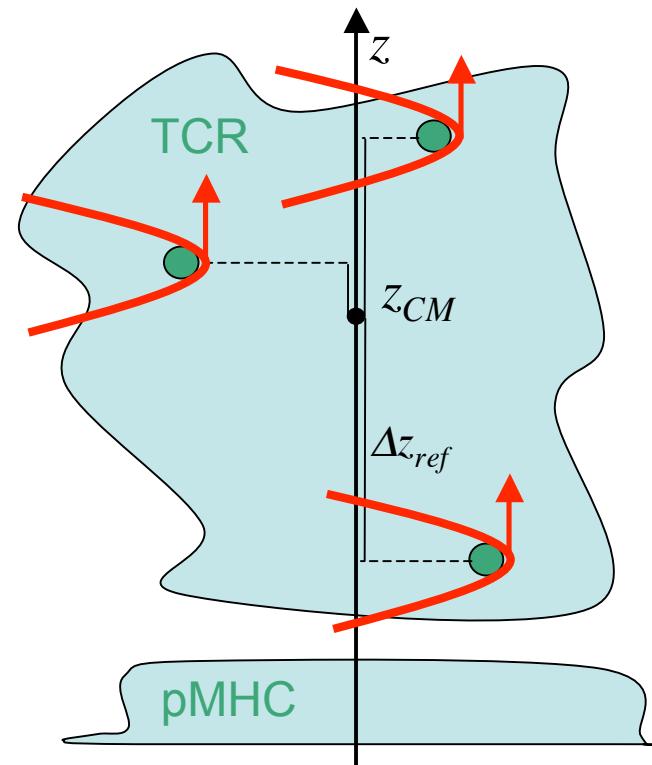
- Structures in solution very similar
- Inconsistent with experimental rates



Rolling

Considered improbable :

- High work necessary
- Multimers in vivo
- Membranes

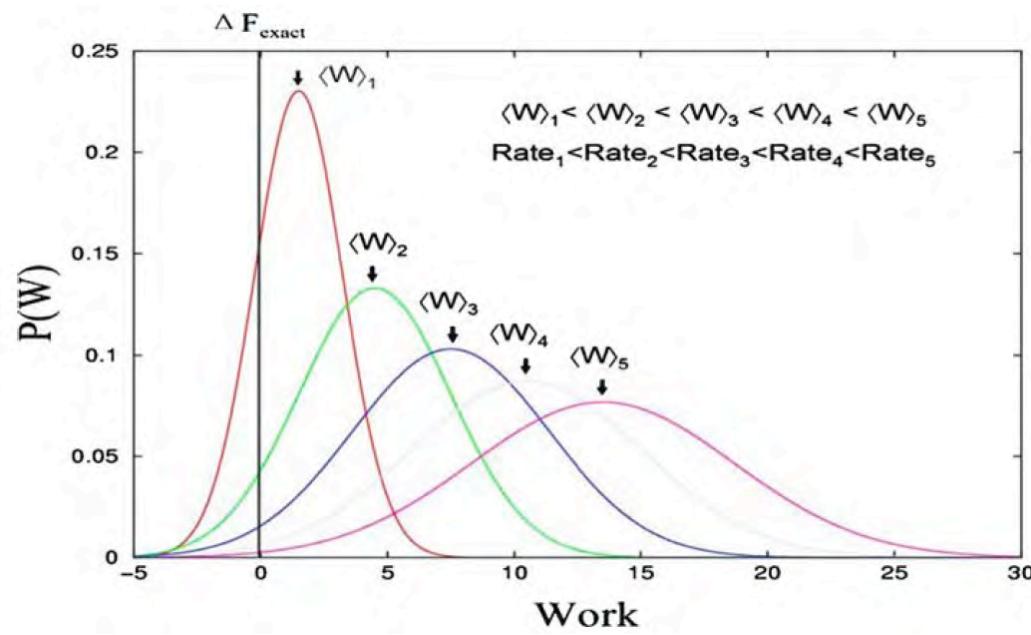
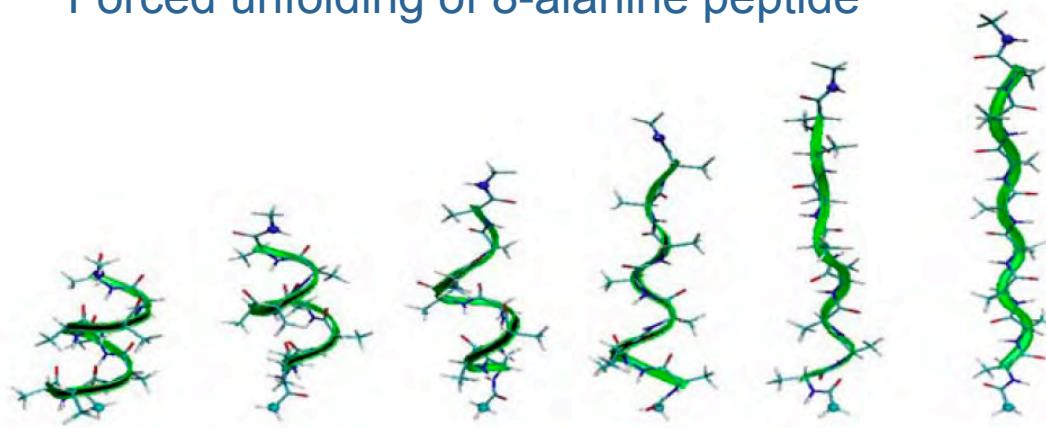


- Action / reaction respected
- Only along z
- Only backbone atoms, not CDR loops

The Jarzynski identity



Forced unfolding of 8-alanine peptide

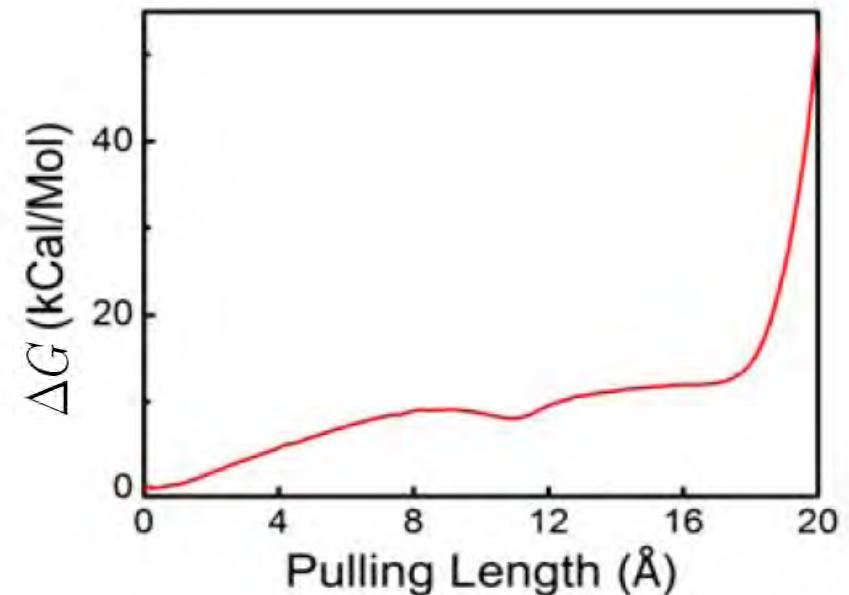


Usual thermodynamics :

$$\langle W \rangle \geq \Delta G$$

Jarzynski identity

$$\langle e^{-\beta W} \rangle = e^{-\beta \Delta G}$$



Thermostats in Molecular Dynamics



- Regulate the temperature :

$$\langle T \rangle = T_0$$

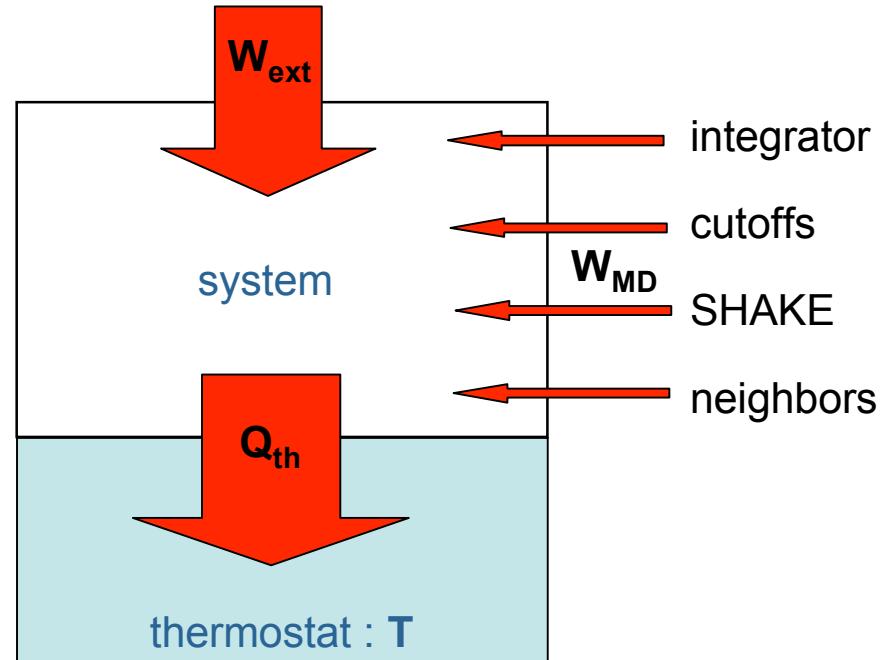
- Generate the right thermodynamic ensemble :

$$\rho(q, p) \propto e^{-\beta H(q, p)}$$

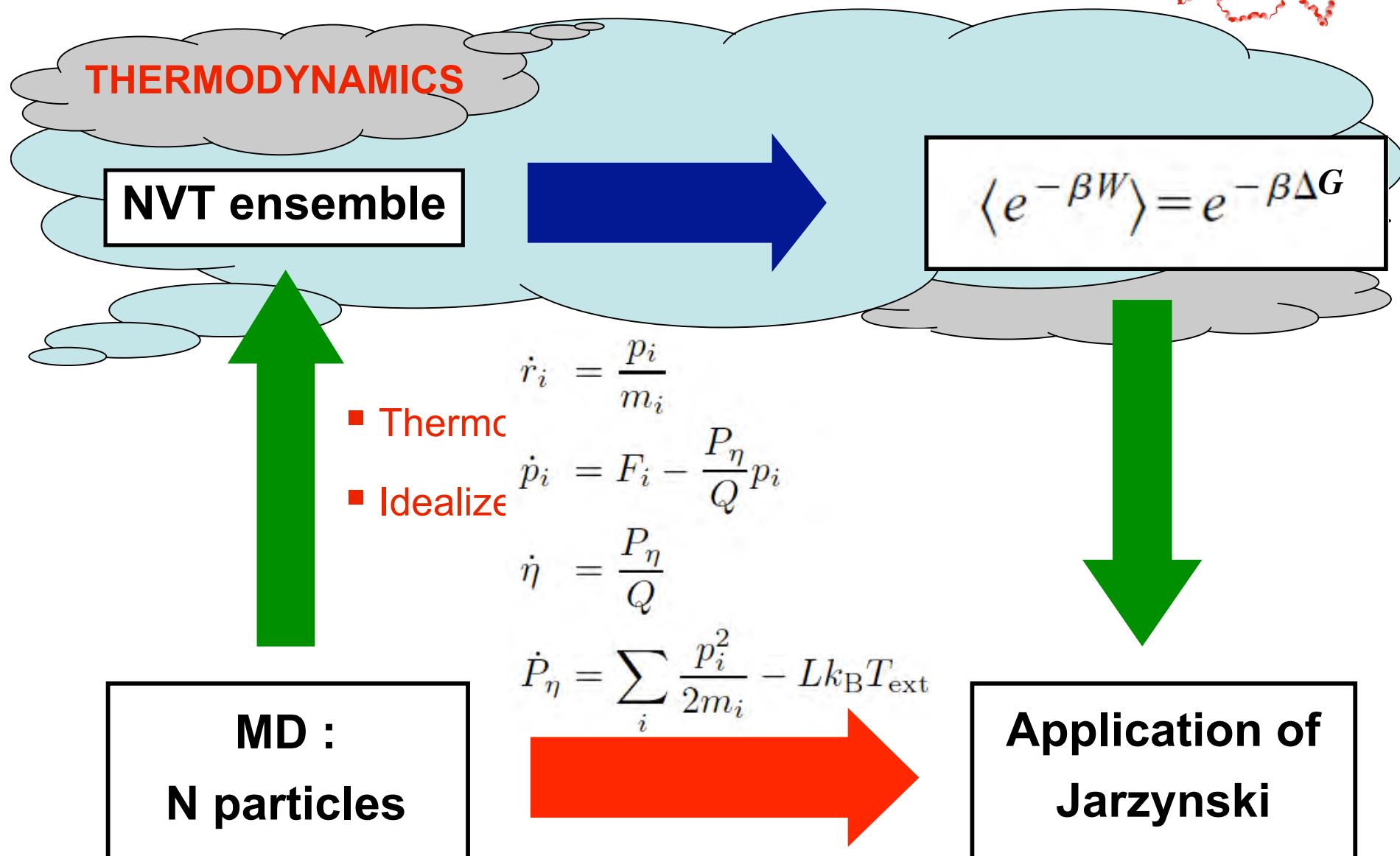
- With the right fluctuations :

$$\langle (\delta T)^2 \rangle = \frac{2 \langle T \rangle^2}{N_{df}}$$

- Thermostats as artificial heat baths : NOT the thermodynamic picture
 - Abstract heat bath replaced by few degrees of freedom
 - Non-Newtonian, strongly coupled dynamics
 - Phase space compression / entropy sink



Jarzynski derived from dynamics



Protocol to apply Jarzynski

$$\langle e^{-\beta W} \rangle = e^{-\beta \Delta G}$$

- The **steering potential energy** prevents direct averaging :

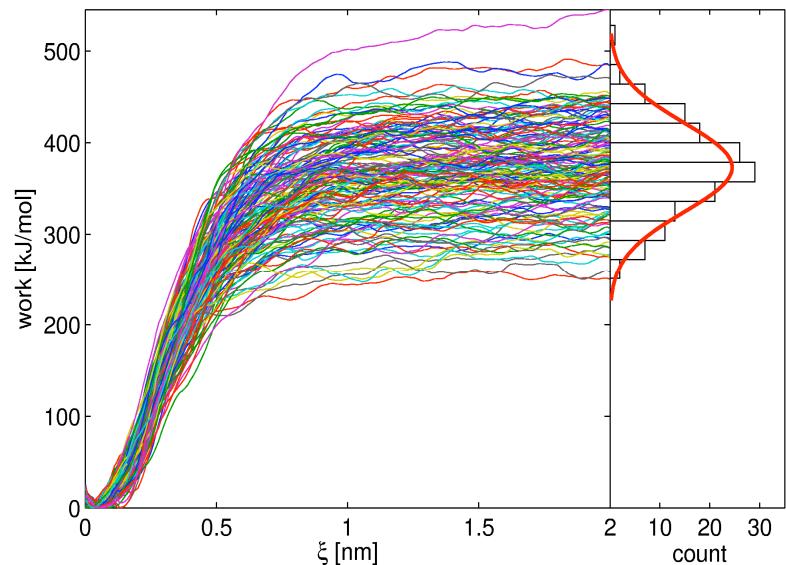
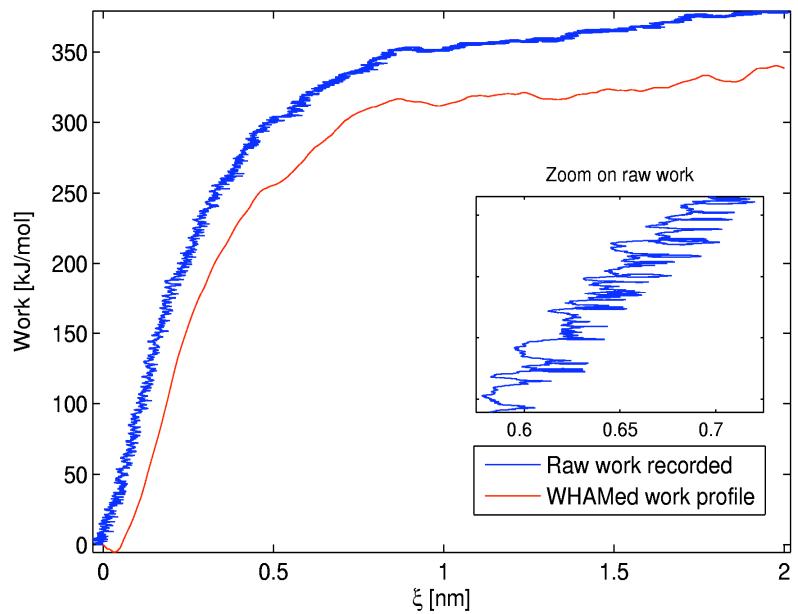
1) Adapted WHAM

- applied to each trajectory separately
- smooth and subtract steering potential

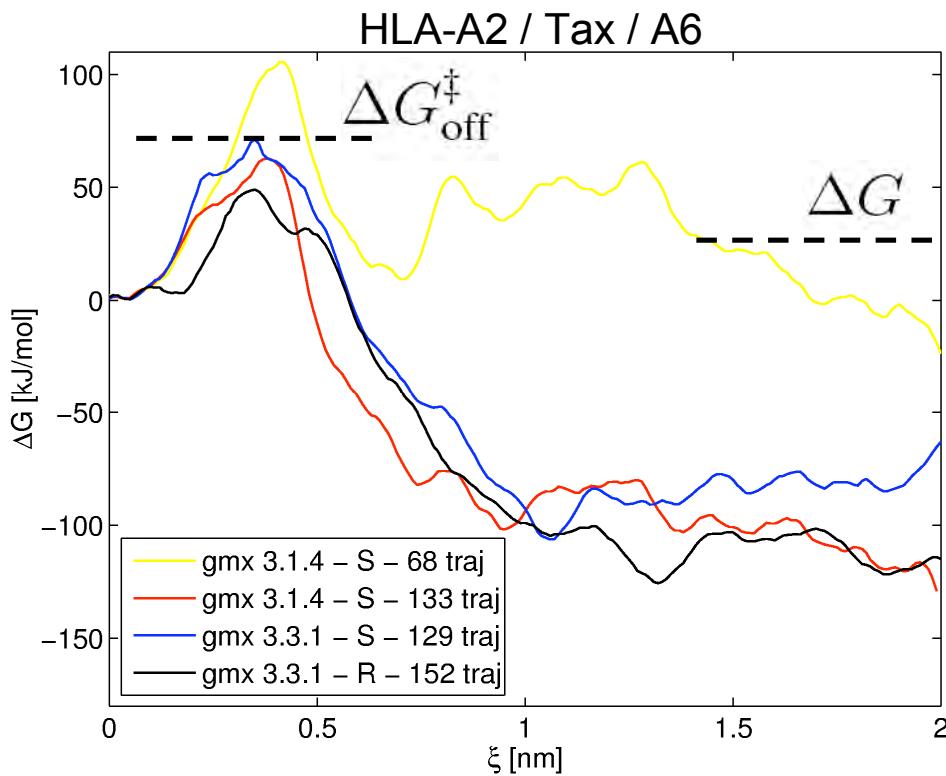
- Exponential average : $\langle e^{-\beta \bar{W}(\xi)} \rangle$
Statistical bias prevents direct evaluation.

2) Cumulant expansion :

$$\Delta G(\xi) = \langle \bar{W}(\xi) \rangle - \frac{\beta}{2} \sigma_{\bar{W}}(\xi) + \dots$$

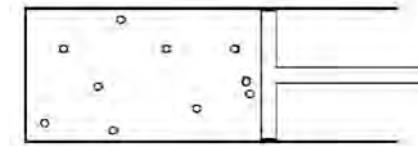


Failure of the Jarzynski method

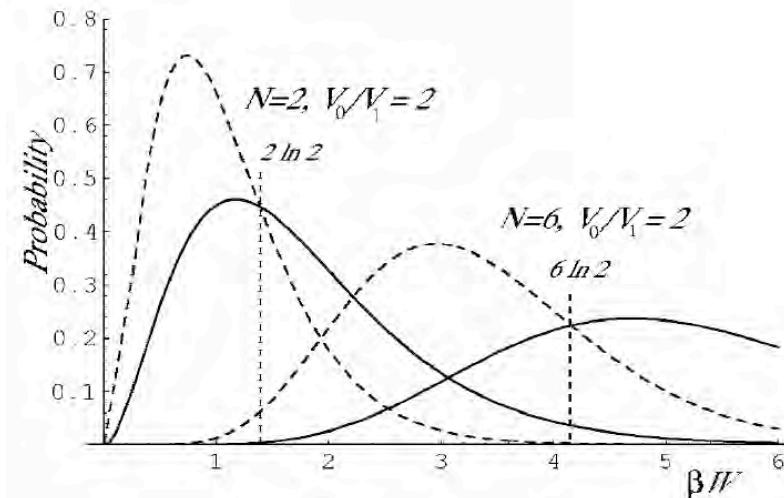


- Converges, but systematic error
- Lower tail seems too heavy
- Gaussian assumption not valid for extrapolation

Analytical case :
Interacting gas compression

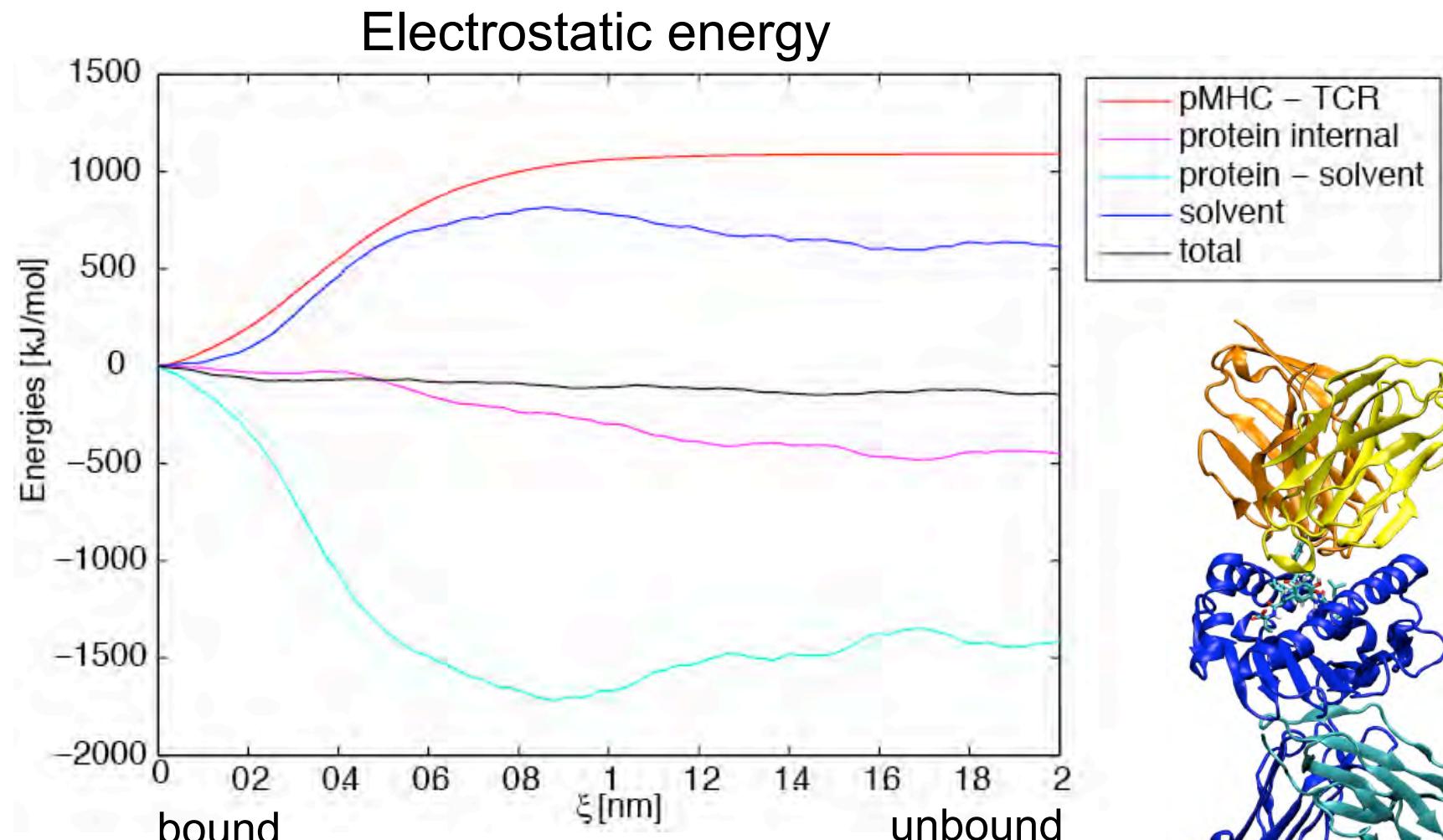


→ Gamma distribution of W :



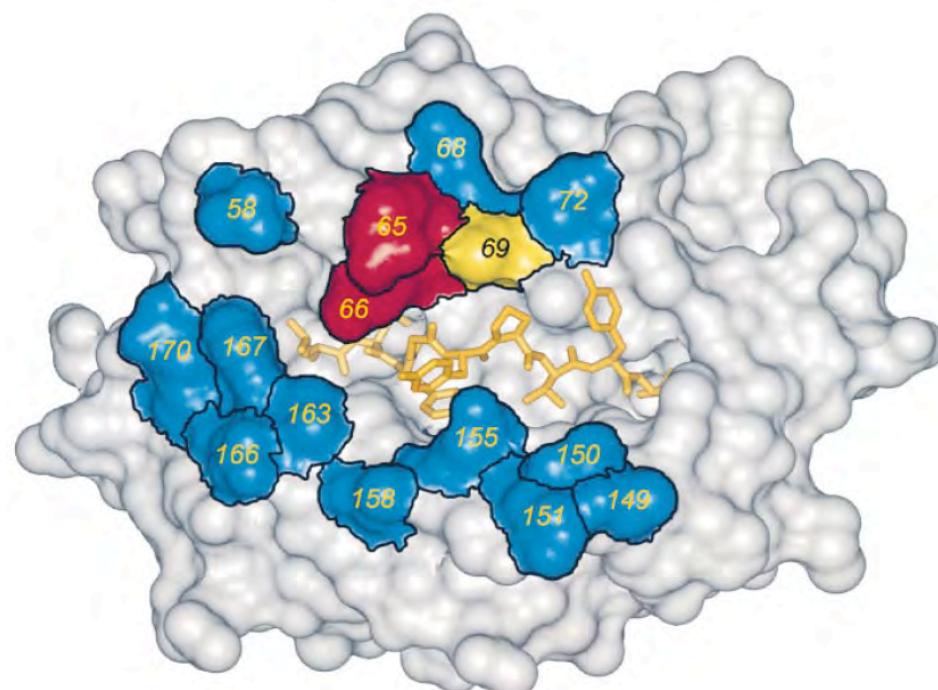
→ Shorter lower tail ...

Interaction Energetics



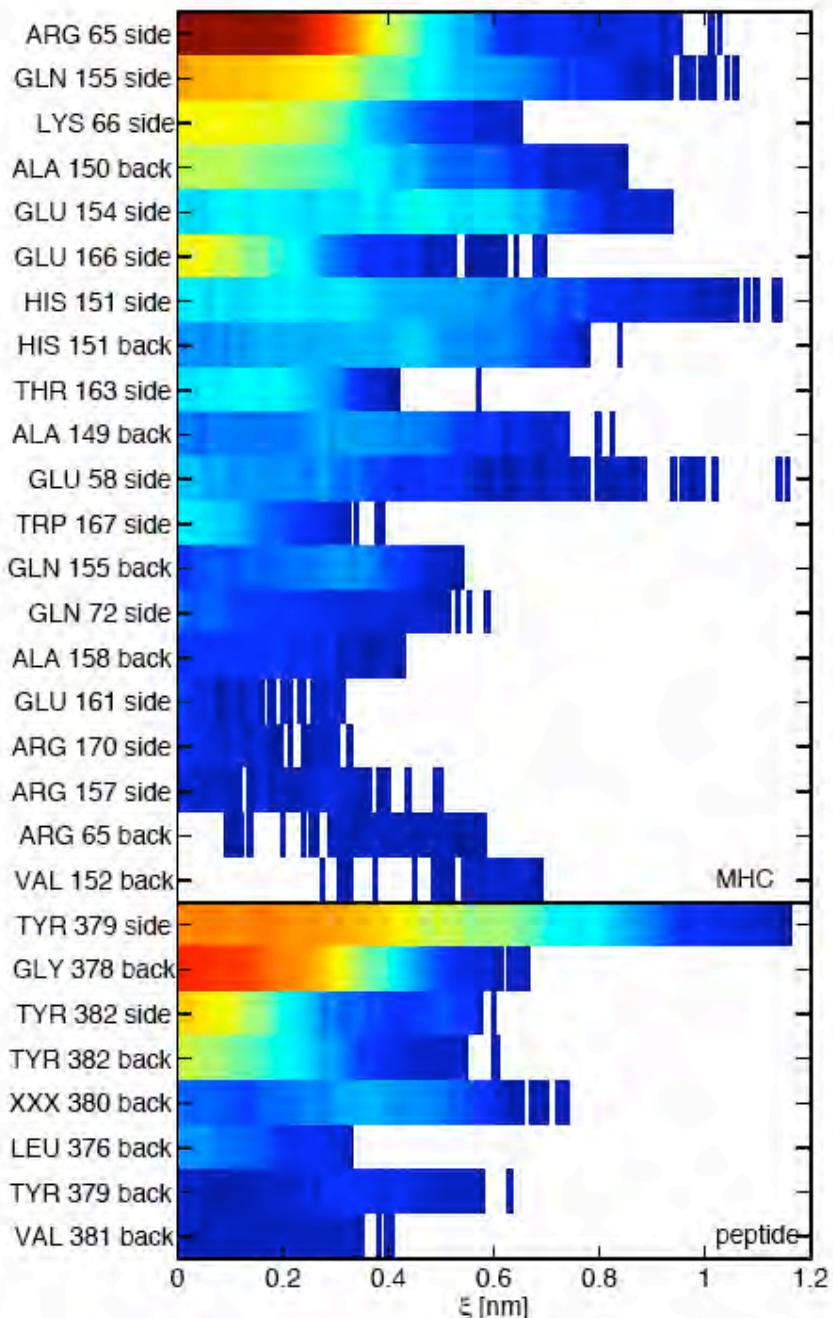
Recognition hotspot

Essential residues for
T-cell activation, identified by
alanine scanning :



Brian M. Baker et al., J. Exp. Med. 193 (2001), no. 5, 551–562.

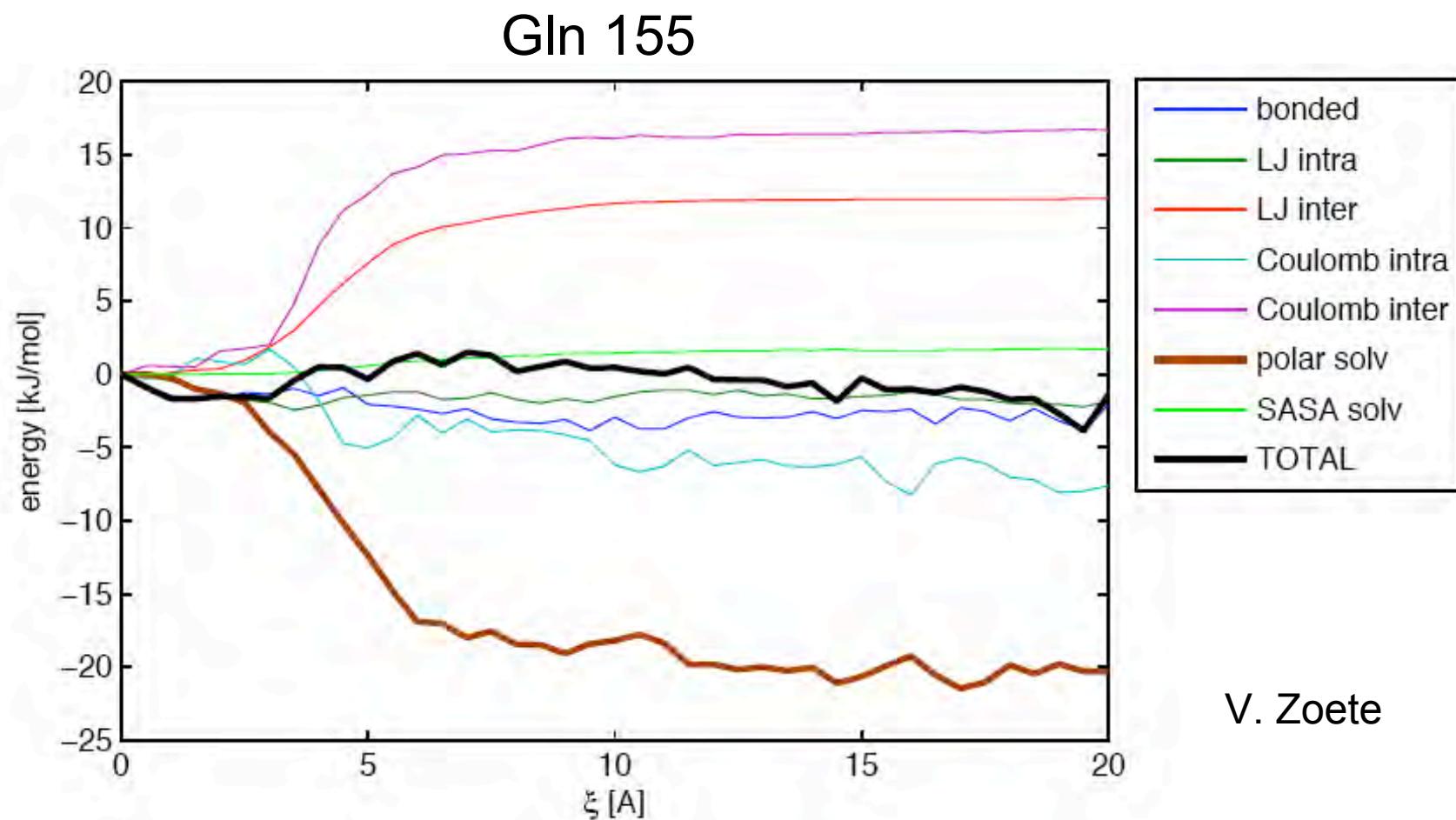
H bonds



MM/GB-SA analysis

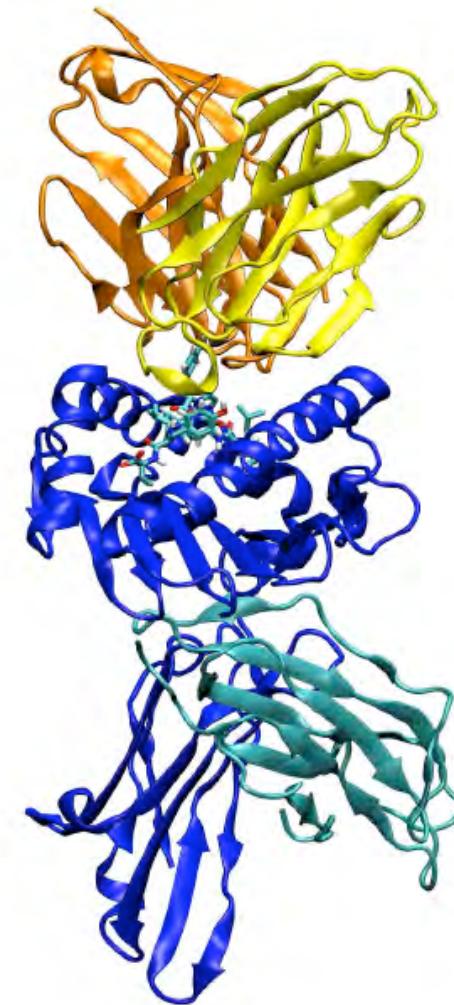
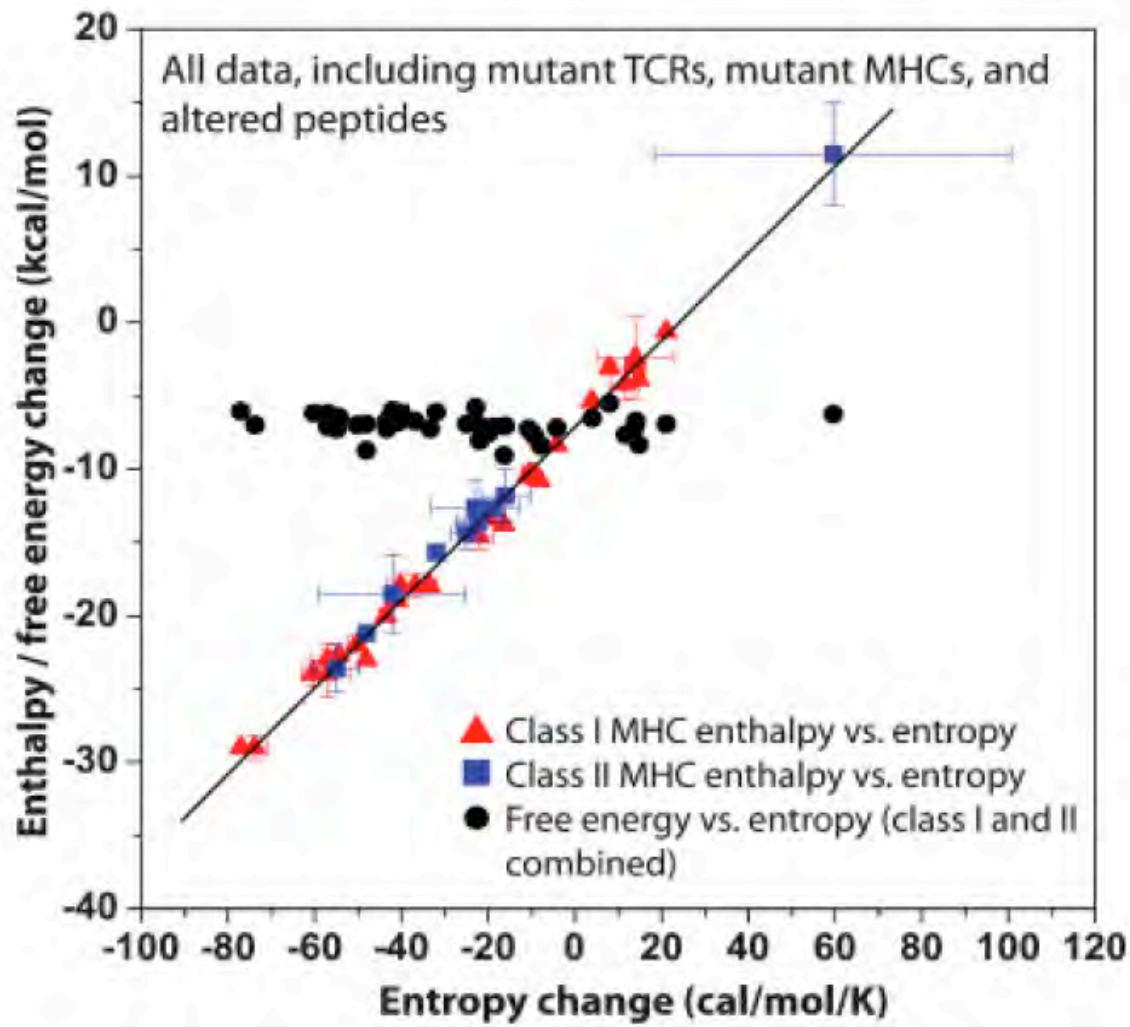


Binding "free energy" decomposition
for individual residues

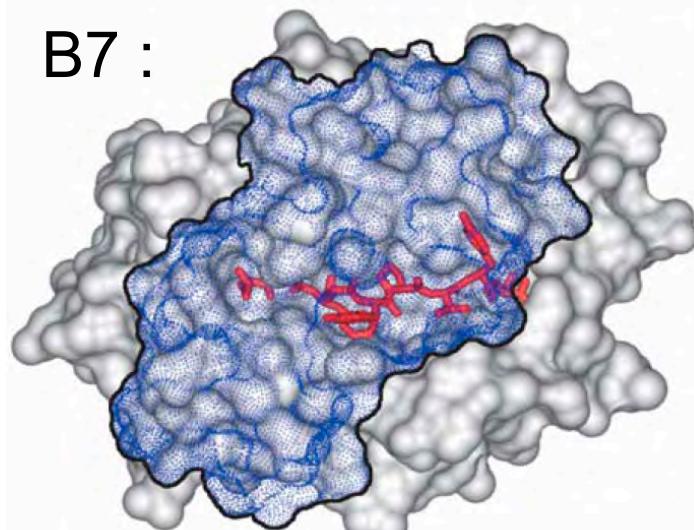
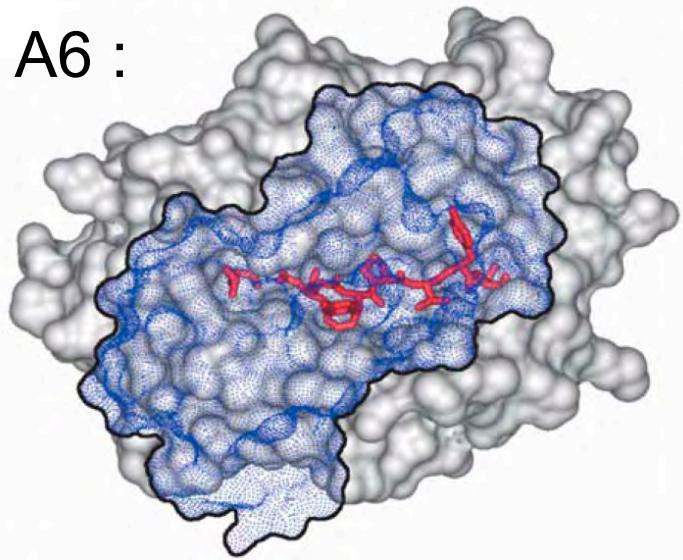


V. Zoete

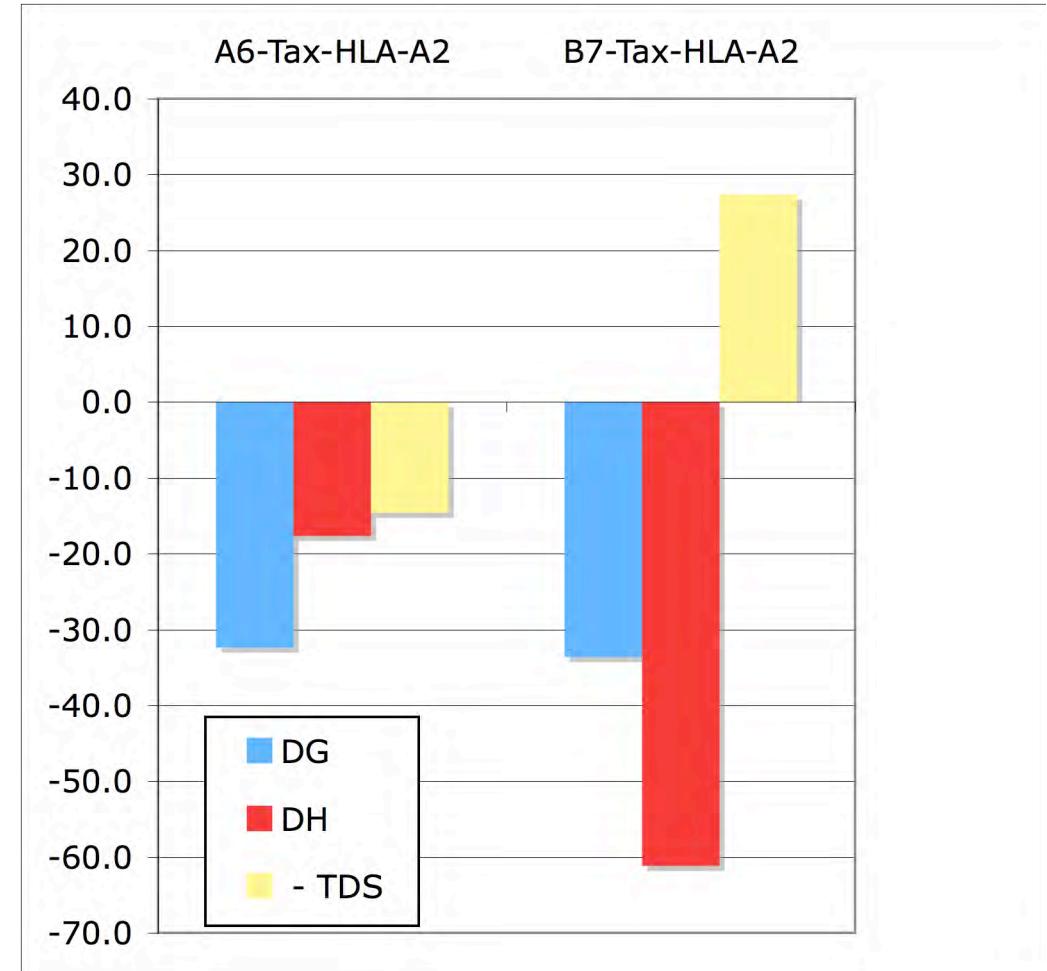
Entropy - enthalpy compensation



The B7 TCR binds the same pMHC



ITC measurements :

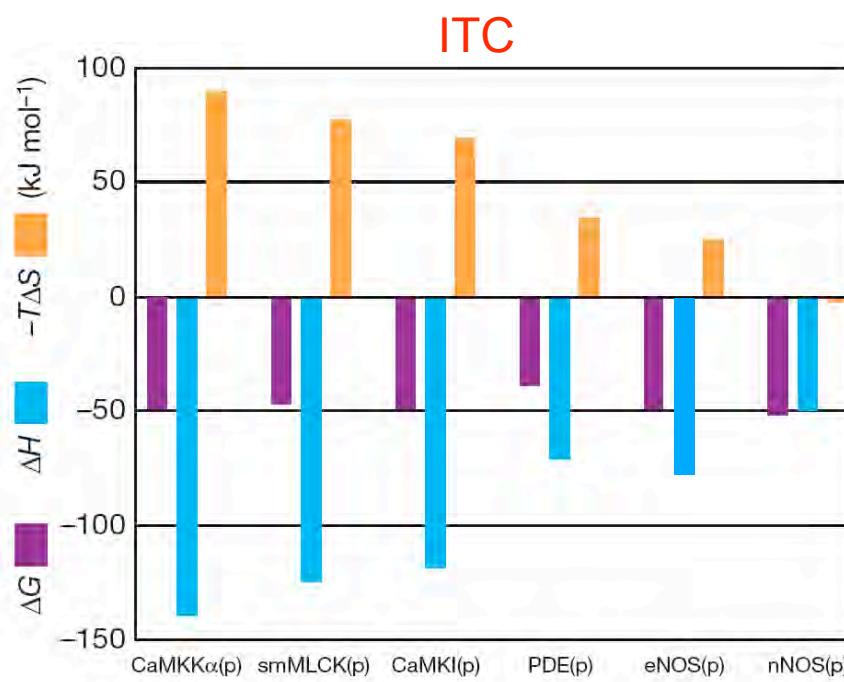


Internal entropy variations

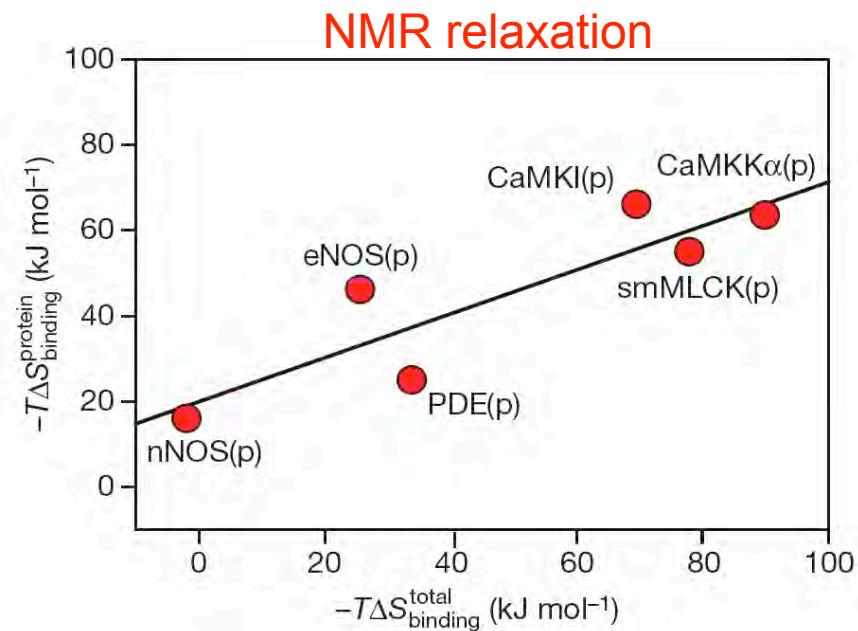


Example : Protein calmodulin and 6 peptide ligands

Same protein binds various partners with similar affinities, but different entropies.



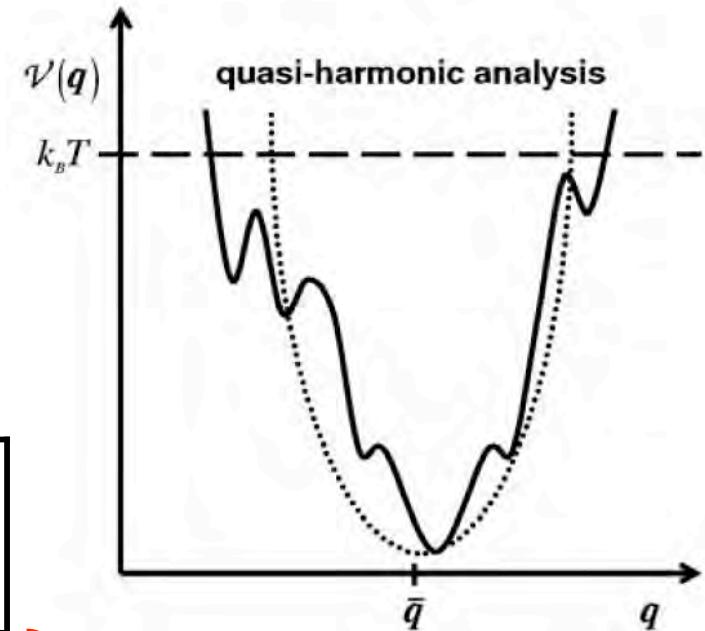
Some of the differences come from changes in **internal** conformational entropy



Quasi harmonic analysis

- Binding ΔS all negative : bias ?
- Difference A6 - B7 meaningful.

	A6 complex ΔS_{QHA} [kJ/mol/K]	B7 complex ΔS_{QHA} [kJ/mol/K]	$B7 - A6$ $-T\Delta\Delta S_{QHA}$ [kJ/mol]
pMHC	-1.30	-1.33	+ 10
TCR	-1.11	-1.18	+ 21
peptide	-0.47	-0.35	- 49
CDR	-1.04	-0.77	- 80



} Total : +31 kJ/mol

- Trend for interface and whole molecules differ
- Overall, the binding entropy seems to be 31 kJ/mol more favorable for A6 than for B7.

Other (rough) entropy contributions

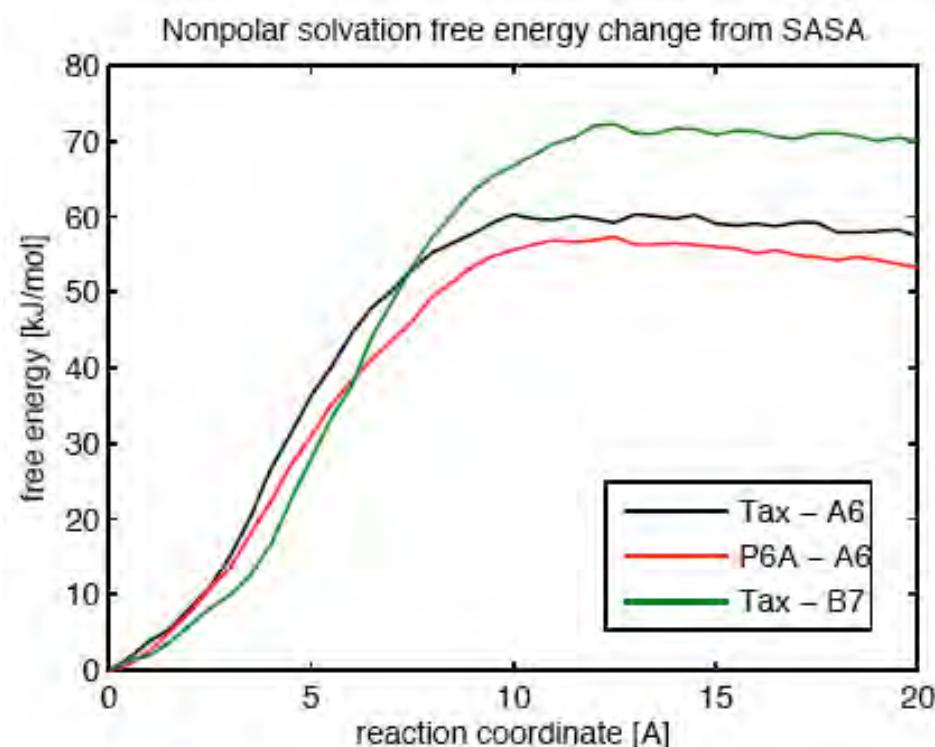


- Translational/rotational entropy

$$\Delta G^{\text{trans/rot}} = k_B T \log \frac{8\pi^2 V_0}{(2\pi)^3 \sin(\theta_0) \sigma_x \sigma_y \sigma_z \sigma_\phi \sigma_\theta \sigma_\psi}$$

-	$T\Delta S_{\text{trans/rot}}$
A6 :	+ 29 kJ/mol
B7 :	+ 35 kJ/mol
$\Delta\Delta$:	+ 6 kJ/mol

- Surface area solvation term



-	$T\Delta S_{\text{solvation}}$
A6 :	-58 kJ/mol
B7 :	-70 kJ/mol
$\Delta\Delta$:	-12 kJ/mol

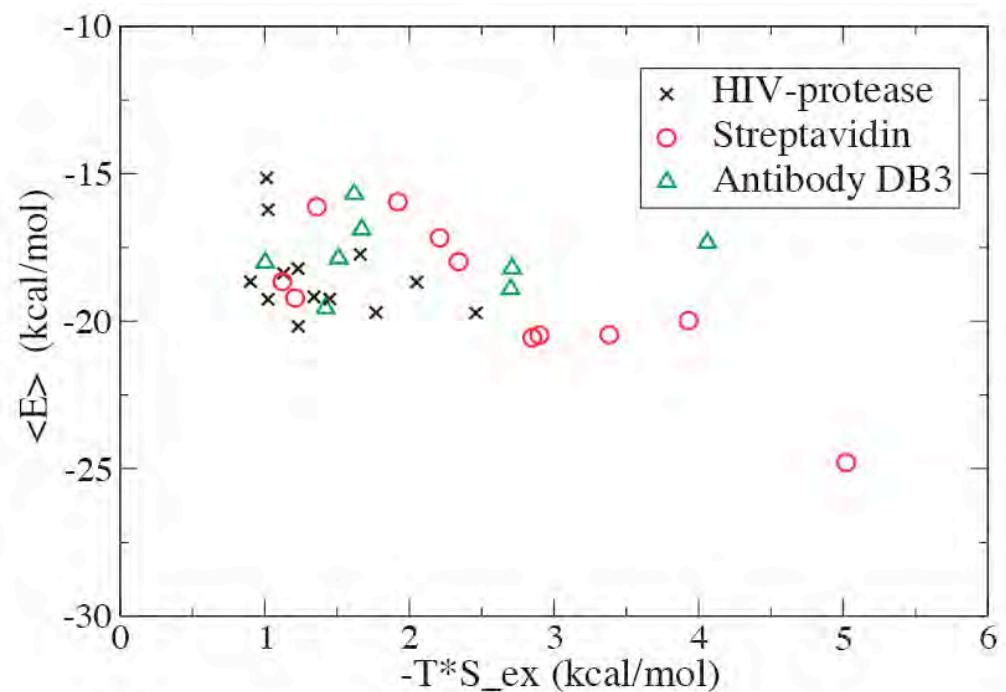
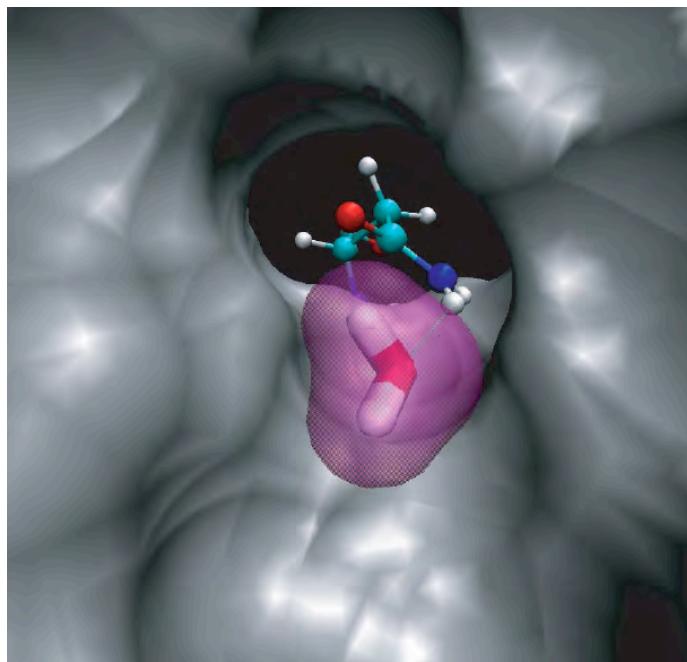
TOTAL including QHA
 $-\Delta\Delta S_{\text{bind}} = +25 \text{ kJ/mol}$
A6 binding is more entropy favored than B7

Experimental: +34 kJ/mol

Specific solvation effects

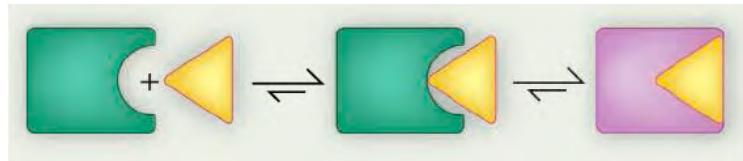


- Protonation effects
- Ion release upon binding (exp: 0.4 for A6 and 0.2 for B7)
- "Dry" hydrophobic pockets
- "Hydrophobic enclosure" effects

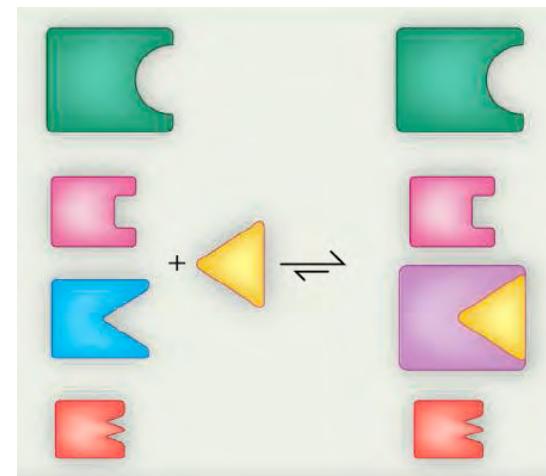


Protein recognition mechanism

- Induced fit :



- Conformer selection :

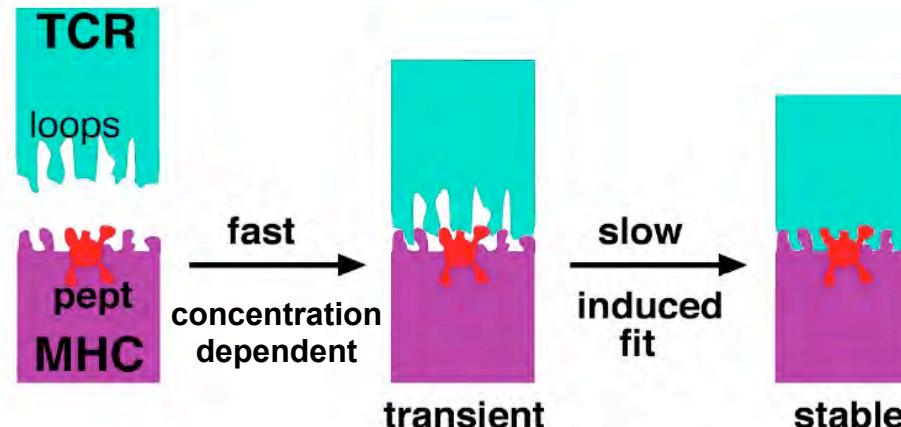


PNAS

Kinetic evidence for a ligand-binding-induced conformational transition in the T cell receptor

Dmitry M. Gakamsky^{†‡}, Erwin Lewitzki[§], Ernst Grell[§], Xavier Saulquin[¶], Bernard Malissen[¶], Felix Montero-Julian^{††}, Marc Bonneville^{¶††}, and Israel Pecht^{†††}

PNAS 104 (2007)

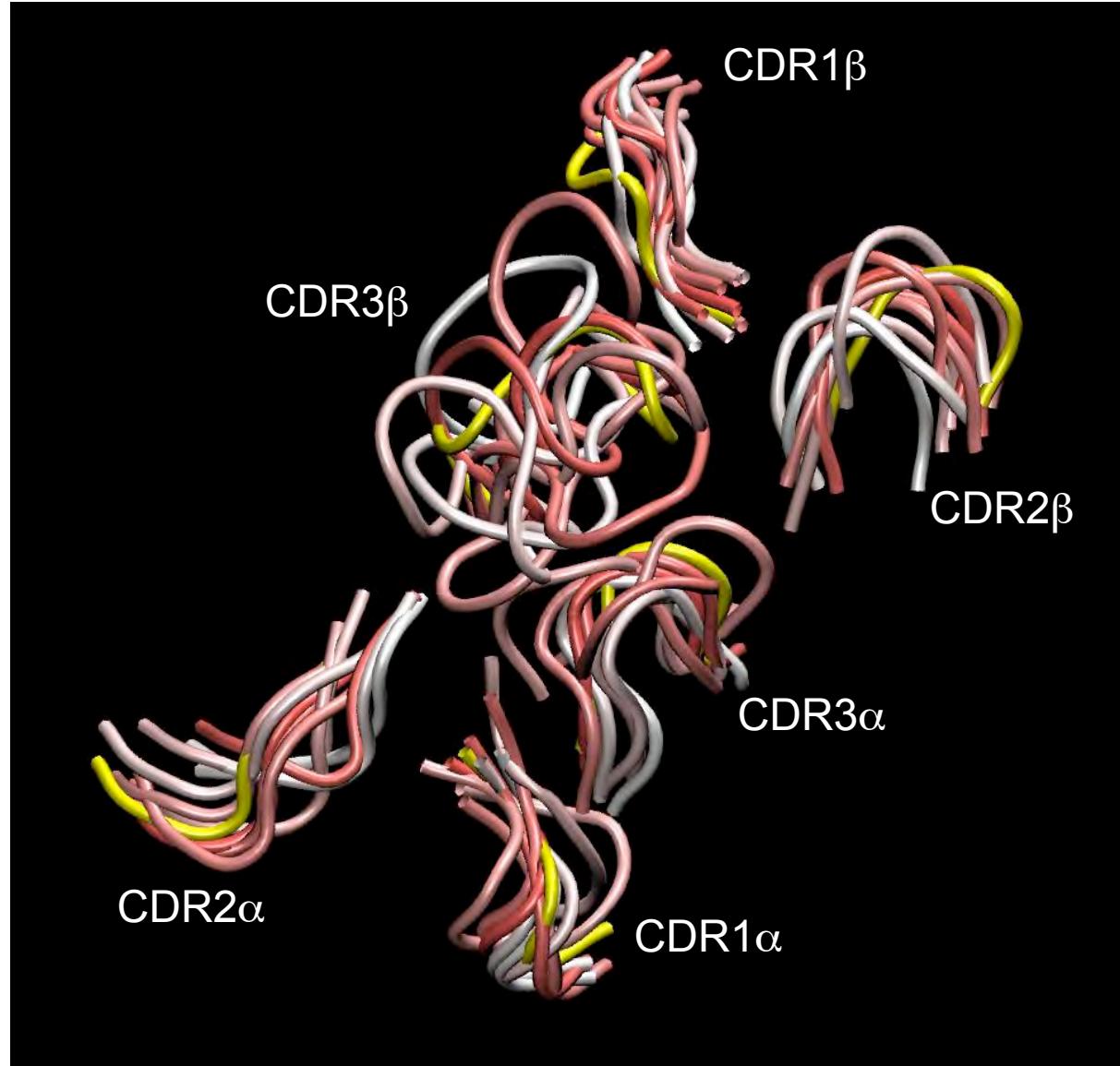


Boehr & Wright

Conformations in solution ?



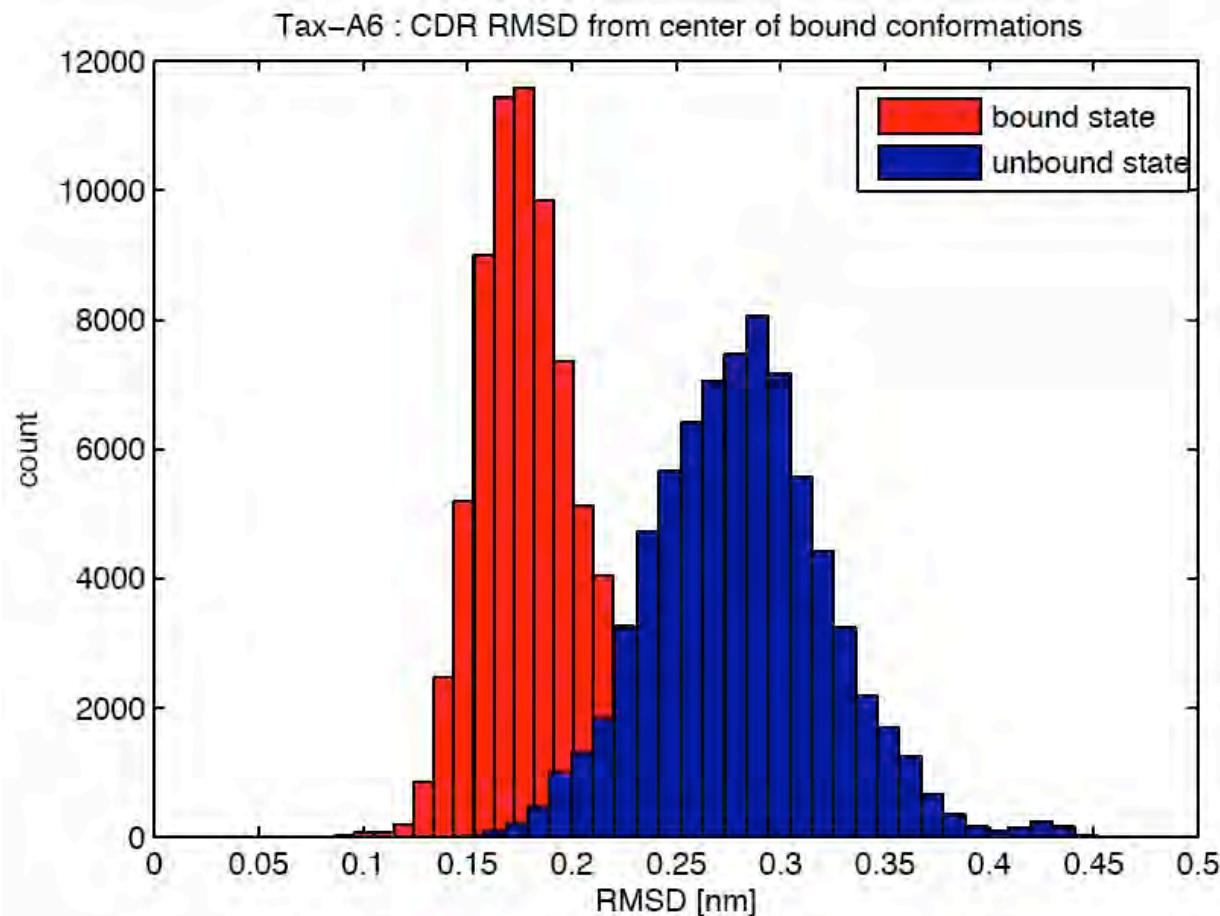
A6 :



crystal
most
populated
cluster

less
populated
cluster

RMSD : overlapping populations



Free TCR samples CDR conformations close to the bound state.
Induced fit does only the last part of the job.



Thanks for your attention !!

Acknowledgements :

- Olivier Michelin
- Vincent Zoete
- Wilfred van Gunsteren
- The VitalIT Computing Facility
 - Roberto Fabbretti
 - Volker Flegel